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Full Length Research Paper

Exploring cucumber extract for skin rejuvenation

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This study was designed to develop a topical skin-care cream water in oil (w/o) emulsion of 3% cucumber extracts versus its vehicle (Base) as control and evaluates its effects on skin-melanin, skin erythema, skin moisture, skin sebum and transepidermal water loss (TEWL). Hydroalcoholıc cucumber *(Cucumis sativus) fruit* extract was entrapped in the inner aqueous phase of w/o emulsion. Base containing no extract and a formulation containing 3% concentrated extract of *C. sativus* was formulated. The odour was adjusted with few drops of lemon oil. Both the base and formulation were stored at different storage conditions for a period of 4 weeks to predict their stability. Different stability parameters that is: Physical stability, centrifugation and pH were monitored at different time intervals. Both the base and formulation were applied to the cheeks of 21 healthy human volunteers for a period of 4 weeks. The expected pharmaceutical stability of creams was achieved from 4 weeks *in vitro* study period. Odour disappeared with passage of time due to volatilization of lemon oil. The base showed insignificant ($p > 0.05$) effects on all skin parameters except sebum that was not significant, whereas the formulation showed statistically significant ($p \leq 0.05$) effects on skin sebum secretion. TEWL and erythema was increased while skin melanin and skin hydration level was decreased by formulation. However these effects were statistically insignificant ($p > 0.05$). The results showed a good stability over 4 weeks of observation period of both base and formulation and the formulation has anti sebum secretion, bleaching and moisturizing effects.

Key words: Cucumber extract, melanin, skin moisture, skin sebum, transepidermal water loss (TEWL).

INTRODUCTION

Emulsions can offer promising applications in pharmaceutical and cosmetic industries. There has been renewed interest in the emulsion as a vehicle for delivering drugs to the body as they frequently enhance the bioavailability of the drugs (Herbert et al., 1988). Water in oil (w/o) emulsions are employed more widely for the treatment of dry skin and emollient applications (Magdy, 2004). Additional value can be given to these formulations by including active ingredients with specific cosmetic effects. Particularly, advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients (Bleckmann et al., 2006). There is a growing interest in natural antioxidants found in plants. Many antioxidatively acting compounds are isolated from natural herbs and extracts and used as potential antioxidants in cosmetics (Naveed, 2001).

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Abbreviations: TEWL, Transepidermal water loss; **w/o,** water in oil; **RH,** relative humidity.

Cucumber extract can be obtained by macerating the crushed fruit in hydro-alcoholic mixture, then filtering and concentrating it on rotary evaporator. Extract so obtained is rich in vitamins, especially vitamin C and A (Claudia, 1992; George, 2001), which have some cosmetic benefits for the skin. In cosmetics, cucumber has an excellent potential for cooling, healing and soothing to an irritated skin, whether caused by sun, or the effects of a cutaneous eruption (Griere, 1992). Cucumber extract is often used for skin problems, wrinkles, sunburn (James, 1997) and as an antioxidant. The commercial aldehyde, trans-cis-2, 6-nonadienal, described as a major volatile compound of cucumber, was characterized as a non-competitive inhibitor against 4-tert-butylcatechol oxidation by mushroom tyrosinase (Fernando et al., 2003). Cucurbitacin D and 23, 24-dihydrocucurbitacin D found in cucumber extract are also responsible for the inhibition of tyrosinase and melanin synthesis (Jian et al., 2005).

The purpose of this study is to develop w/o emulsion (cream) containing the extract of cucumber and measure its effects on different physiologic functions of skin like melanin, erythema, skin moisture, skin sebum, pH of skin and transepidermal water loss (TEWL).

MATERIALS AND METHODS

Identification of plant

The identification of cucumber (*Cucumis Sativus)* was performed at Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan. The specimen was deposited in the herbarium of Pakistan agricultural research council (PARC), the voucher number is: pharm.3986/Feb 2001.

Materials

Cucumber was purchased locally. Paraffin oil (density 0.85) was obtained from Merck (Germany). Abil-EM 90 (Cetyl Dimethicone copolyol with HLB 5) was purchased from Franken Chemical (Germany); Lemon oil was obtained from Chemoflor Manufacturing Corporation Pakistan.

Apparatus

The apparatus used include: Centrifuge machine (Hettich EBA 20, Germany), cold incubator (Sanyo MIR-153, Japan), conductivity-meter (WTW COND-197i, Germany), corneometer MPA 5, mexa-meter MPA 5, sebumeter MPA 5, TEWA meter MPA 5 (Courage + Khazaka, Germany), digital humidity meter (TES Electronic Corp, Taiwan), electrical balance (Precisa BJ-210, Switzerland), homo-genizer (Euro-Star, IKA D 230, Germany), hot incubator (Sanyo MIR-162, Japan), pH-meter (WTW pH-197i, Germany), refrigerator (Dawlance, Pakistan) and rotary evaporator (Eyela, Co. Ltd. Japan).

Preparation of emulsions

In this study, w/o emulsions were prepared by the addition of aqueous phase to the oily phase with continuous agitation (Akhtar et al, 2010). Oily phase consisted of paraffin oil (16%) and surfactant ABIL-EM 90 (4%) was heated up to 75±1°C. At the same time, aqueous phase consisting of water (q.s) was heated to the same temperature and then the cucumber extract (3%) was added to it. After that, aqueous phase was added to the oil phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for about 15 min until complete aqueous phase was added; 2 to 3 drops of lemon oil were added during this stirring time to give good fragrance to the formulation. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization, for a period of 5 min, and then the speed of the mixer was further reduced to 500 rpm for 5 min for complete homogenization; until the emulsion cooled to room temperature.
Base was also prepared by the same above method and with same ingredients but without cucumber extracts.

Pharmaceutical stability tests

Stability tests were performed at 8 ± 0.1°C (in refrigerator), 25 ± 0.1°C, 40 ± 0.1°C and 40 ± 0.1°C (in incubator) with 75% relative humidity (RH). Physical characteristic of emulsions, that is, color, creaming and liquefaction, were noted at various intervals for 28 days.

Study design for product evaluation on skin

One-sided blind study was designed with placebo control in the month of August. 21 healthy human volunteers who signed the informed consent, with age range 20-35 years were selected. All the readings were performed at 21±01°C and 40±2% relative humidity conditions.

The experiments were carried out on the cheeks of volunteers. Patch test was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. Each volunteer was provided with two creams. One cream was base and the other was formulation. Each cream was marked with “right” or “left” indicating application of that cream to the respective cheek. The creams were applied twice a day by the volunteers themselves as instructed for 60 days. Every individual was instructed to come on 1st, 2nd, 3rd and 4th week for the skin sebum measurements.

Ethical standards

This study was approved by the Board of Advance Study and Research (BASR), The Islamia University of Bahawalpur and institutional ethical committee in compliance with NIH Principles of Laboratory Animal Care 1985. The Reference No is 1457/07.

Burchard tests (Patch tests)

Patch tests were performed on the forearms of each volunteer. The patch (Bandage disc) for the right forearm was saturated with 1.0 g of base while the patch for left forearm was saturated with 1.0 g of formulation. Each was applied to the 5 cm X 4 cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. The patches were removed after 48 hours and the forearms were washed with physiological saline. After 48 hours, scores were recorded for the presence of erythema (skin redness) using a scale with 4 points from 0 to 3, where 0 stands for absence of erythema, 1 for mild erythema, 2 for mode-rate erythema while 3 stands for severe erythema. Each volunteer

Table 1. Score given by volunteers to base and formulation on the basis of itching/irritation.

Skin-care cream	Score			
	0	1	2	3
Base	14	5	2	0
Formulation	16	3	2	0

was asked to note their irritation/itching towards the patches and then assign a score from the same scale. Average score with respect to volunteers is given in Table 1.

Panel test

Every individual was provided with a Performa preparedpreviously to test the sensory values of cream. This form consisted of seven parameters to be evaluated and every parameter was assigned 11 values from –5 to +5 indicating very bad to very good, respectively. This form was asked to be completed independently by each individual on day 28. Average points were calculated from the points assigned by each volunteer for each question for both of the creams, base and formulation (Figure 6).

Average points for the first question, that is, ease of application of creams were found to be 4.00 and 4.20 for the base and formulation, respectively. This indicated that base and formulationcan easily be applied on the skin. Average points regarding spread-ability were 4.40 for base and 4.10 for formulation which meant that the formulation spread on skin better than the base. Average points for feel on application were 3.70 for the base and 3.30 for formulation. This indicated that base was felt well on the skin than formulation. Average points for the sense in long-term application of creams were 3.50 and 3.60 for the base and formulation, respectively. This showed that formulation produced more pleasant feeling on application to skin than base. There was no irritation on the skin in both cases, that is, base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was 3.30 for the base and 3.20 for formulation. This was expected since the base and formulation contained same quantity of paraffin oil. Similarly, the formulation led to more softness of the skin than base as the average point was 4.30 for base and 4.60 for formulation.

It was found from paired sample t-test that there was a non-significant difference between the average points of sensitivity for base and formulation. It was concluded that there was no variation between base and formulation regarding the sensory evaluation. Both creams behaved similarly from the sensory point of view.

The percentage changes for the individual values of different parameters, taken every week, of volunteers were calculated by the following formula:

$$\text{Percentage Change} = [(A - B) / B]*100$$

Where, A, Individual value of any parameter of 1^{st}, 2^{nd}, 3^{rd}, or 4^{th} week; B, zero hour value of that parameter.

Statistical analysis

The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, elasticity and pH) were analyzed using SPSS 12.0 on computer (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals). 5% level of significant was applied.

RESULTS

Pharmaceutical stability tests

Organoleptic tests (color, liquefaction and phase separation)

In this study, base and formulation were divided in to four samples separately and these samples were kept at 8°C in refrigerator, at 25, 40 and 40°C + 75% RH (relative humidity) in stability chambers. They were observed organoleptically with respect to change in color, liquefaction and phase separation for a period of 28 days at definite time intervals.

Centrifugation

Centrifugation tests for base and formulation kept at different storage conditions were performed at 5000 rpm for 10 min and phase separation was observed for 28 days at different time intervals. No phase separation after centrifugation was found in any of the samples of base and formulation.

Electrical conductivity

Electrical conductivity values for base and formulation kept at different storage conditions for 28 days were determined**.** No change in electrical conductivity was found in any sample of base and formulation. The value of electrical conductivity always remained zero.

pH tests

pH values of the base and formulation kept at different storage conditions for 28 days have been determined and reported in Table 2.

Dermatological tests

The percentage of changes in the measured skin-melanin, skin erythema, skin moisture, skin sebum, TEWL following the application of the base and the formulation on the cheeks of human volunteers is demonstrated in

Table 2. pH values of base and formulation kept at 8, 25, 40 and 40°C + 75% RH.

Time	8°C		25°C		40°C		40°C+75% RH	
	B	F	B	F	B	F	B	F
0 h	5.38	5.89	5.38	5.89	5.38	5.89	5.38	5.89
12 h	5.27	5.78	5.53	5.93	5.41	5.72	5.17	5.69
24 h	5.89	5.81	5.64	5.86	5.66	5.64	5.36	5.72
36 h	5.96	5.84	5.47	5.61	5.83	5.80	5.51	5.79
48 h	5.67	5.74	5.81	5.71	5.43	5.42	5.24	5.61
72 h	5.77	5.69	5.62	5.76	5.48	5.29	5.38	5.62
7 days	5.28	5.90	5.83	5.86	5.21	5.67	4.86	5.55
14 days	5.55	5.81	5.80	5.72	4.94	5.65	4.90	5.34
21 days	5.34	5.80	5.22	5.71	4.66	5.72	4.26	5.57
28 days	5.20	5.24	4.60	5.70	4.31	5.10	4.03	5.26

Where, B, Base; F, formulation; RH, relative humidity.

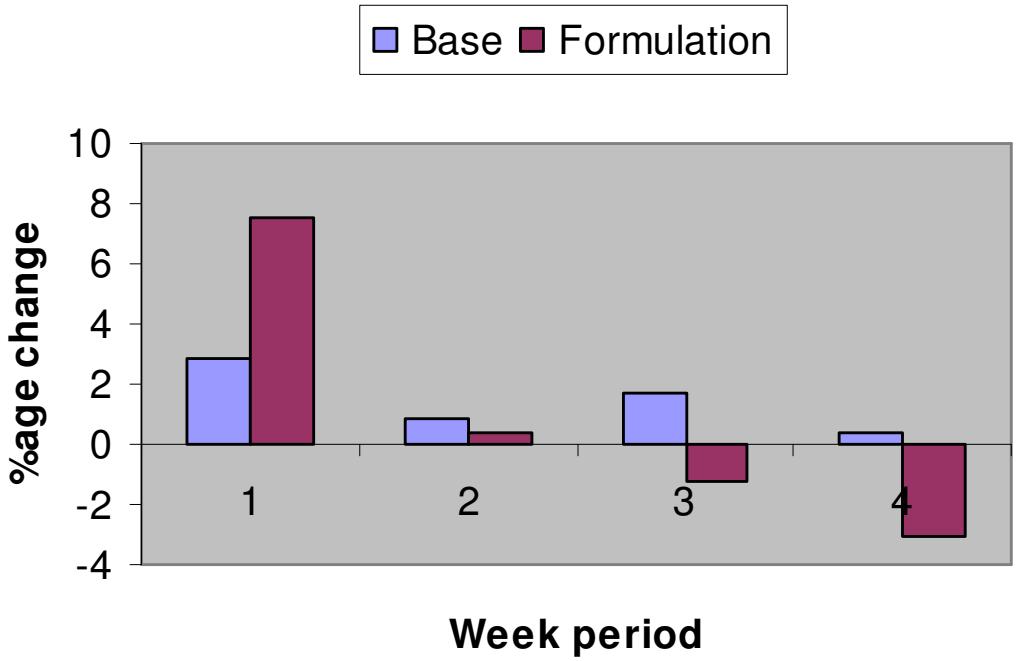

Figure 1. Percentage of change in skin melanin content after application of base and formulation. Where, 1, 1st week; 2, 2nd week; 3, 3rd week; 4, 4th week.

Figures 1, 2, 3, 4, 5 and 6, respectively.

DISCUSSION

Color

The freshly prepared base and the formulation were elegant white in color. No change occurred in color up to the observation period of 28 days. This showed that the emulsions were stable at different storage conditions, that is, 8, 25, 40 and 40°C + 75% RH throughout the 28 days study period.The observed no change in the color of base and formulation may be attributed to different factors contributing to emulsion stability, such as, the components of oil phase that is, paraffin oil which is a colorless, transparent, tasteless, non-fluorescent liquid and is mix-ture of hydrocarbons (Henriette, 1995), Abil-EM90 which is a clear, colorless and nontoxic liquid emulsifier (Raymond et al., 2003). As cucumber extract contains poly amine spermidine, which has bacterial growth inhibitory effect (Khawola and Khuther, 1987), it

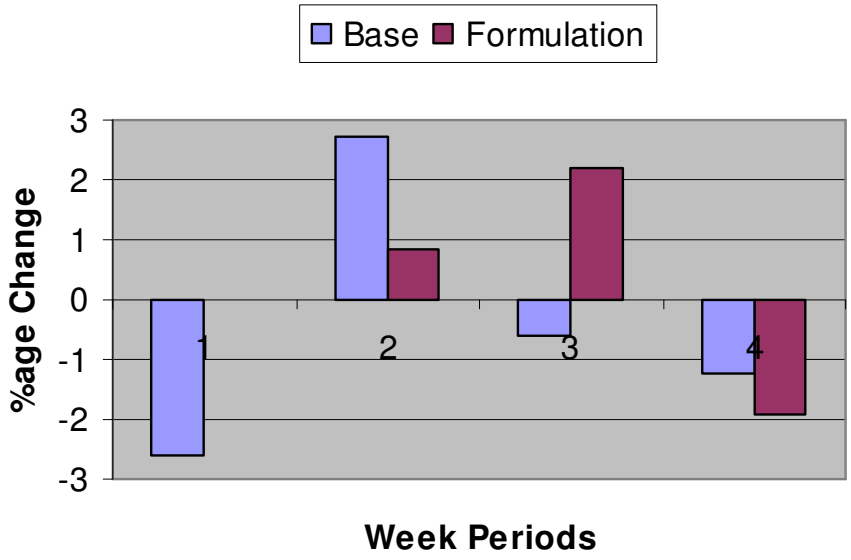

Figure 2. Percentage of change in skin erythema content after application of base and formulation. Where, 1, 1st week; 2, 2nd week; 3, 3rd week; 4, 4th week.

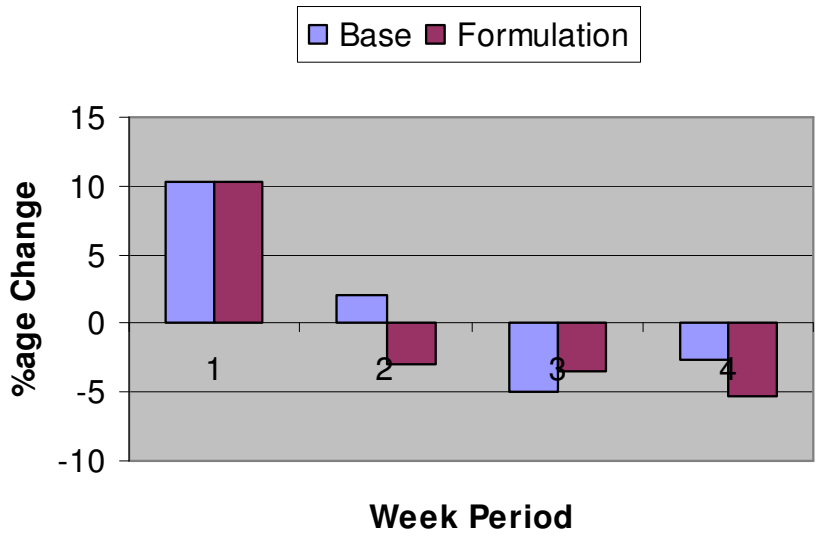

Figure 3. Percentage of change in skin moisture content after application of base and formulation. Where, 1, 1st week; 2, 2nd week; 3, 3rd week; 4, 4th week.

may protect the formulation from microbial growth which might produce such substances which are able to change the color of the formulation during the storage time.

Liquefaction

The viscosity of emulsions plays an important role in their flow properties and is a useful process indicator of emulsion quality (Nasirideen et al., 1998; Ronald and Thomas, 1994). As soon as an emulsion has been prepared, time and temperature-dependent processes occur to affect its separation leading to the decreased viscosity which results in increased liquefaction (Herbert et al., 1988).

No liquefaction was observed in any of the sample of base and formulation kept at 8 and 25°C during whole observation period of 28 days. Slight liquefaction was observed in the sample of base kept at 40°C on the 28thday. Liquefaction was also observed in sample of base kept at 40°C + 75% RH from 21st day of observation but there was no further increase in liquefaction till the end of the study period. On the other

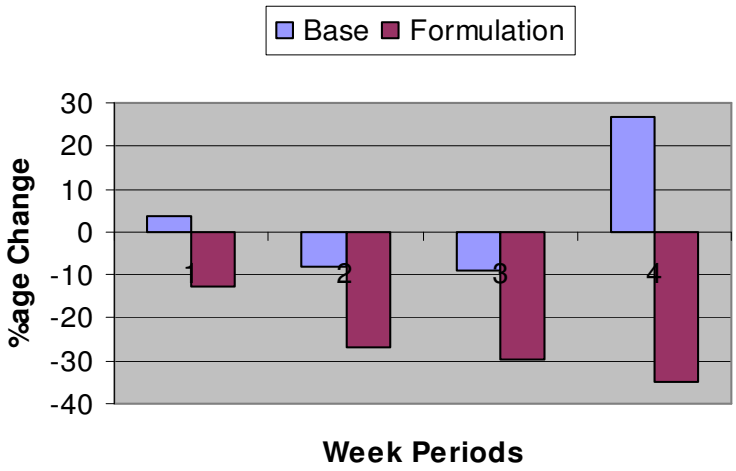

Figure 4. Percentage of change in skin sebum after application of base and formulation. Where, 1, 1st week; 2, 2nd week; 3, 3rd week; 4, 4th week.

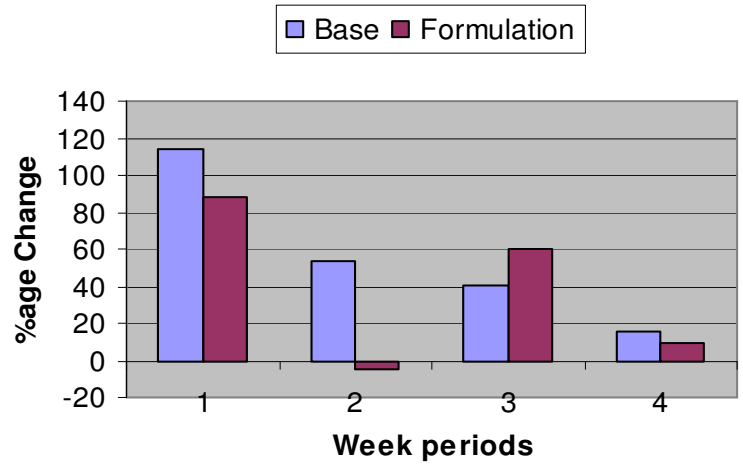

Figure 5. Percentage of change in values of trans epidermal water loss (TEWL) after application of base and formulation. Where, 1, 1st week; 2, 2nd week; 3, 3rd week; 4, 4th week.

hand, a slight liquefaction was observed in formulation samples kept at 40°C + 75% RH on the 28th day of observation. In w/o emulsion, the cream results from sedimentation of water droplets and forms the lower layer. According to the Stokes' law, the rate of creaming is inversely proportional to the viscosity of the dispersion medium. So as creaming increases, the viscosity of the base and formulation gradually decreases with increasing temperature resulting in liquefaction (James and James, 2004).

Phase separation

Creaming is due to differences in density between the two phases under the influence of gravity which leads to phase separation (Derrick, 2000). Coalescence is one of the possible mechanisms of destruction of emulsions, which occur when the energy of adhesion between twodroplets is larger than the turbulent energy causing dis-persion (Abdurahman and Rosli, 2006).

The samples of base were stable at 8 and 25°C but slight separation was observed visually at 40 and 40°C+ 75% RH on the 28th day of observation. In the case of formulation, no phase separation was observed in any of the samples kept at 8, 25, 40 and 40°C+ 75% RH up to observation period of 28 days. This indicated that the formulation was relatively more stable than base at higher temperatures considering phase separation as a parameter of stability. Slight phase separation in case of

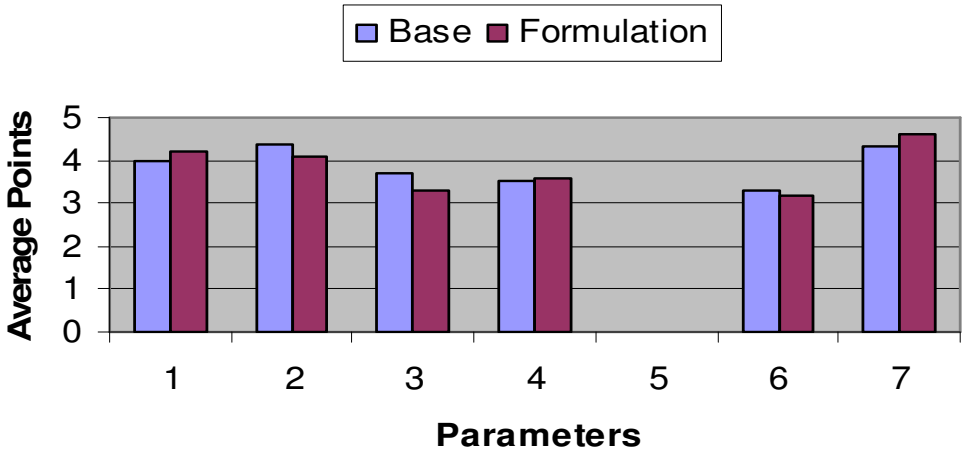

Figure 6. Average values for panel test. Where, 1, Ease of application; 2, spreadability; 3, sense just after application; 4, sense in long term; 5, irritation; 6, shine on skin; 7, sense of softness.

base at higher temperatures may be attributed to the movement of small number of surfactant molecules from interface to the surface (Onuki, 1993) which is much easier when the emulsion has a lower viscosity. Depending on conditions, emulsions may be more stable at lower temperature due to increased phase viscosity (Derrick, 2000).

Centrifugation test

Centrifugation is based on the principle of using centrifugal force to separate two or more substances ofdifferent density, for example, two liquids or a liquid and a solid. In addition, it is an extremely useful tool for evalua-ting and predicting the shelf life of emulsions (Herbert et al, 1988).

In this study, centrifugation test was performed for the samples of the base and formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No phase separation on centrifugation was seen in any of the samples up to the 28th day. This indicated that the emulsions were stable at all the storage conditions for 28 days. It may be said that proper homogenization speed during emulsion formulation prevented the base and the formulation breakage during stress testing (Abdurahman and Rosli, 2006).

Electrical conductivity

Conductivity differences arises when an emulsion creams and the proportion of oil increases in the upper part of emulsion and the proportion of water increases in the lower part of emulsion (James et al., 2000). In this study, conductivity test was performed for all the samples of base and the formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No electrical conductivity was seen in any of the samples.

pH tests

The pH is a significant parameter as far as the effectiveness of the cream is concerned. The pH of human skin typically ranges from 4.5 to 6.0 (Jennifer et al., 2003) and 5.5 is considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH closer to this range.

In this study, the pH of freshly prepared base and formulation was 5.38 and 5.89, respectively, which is within the range of skin pH. The pH values of the samples of base kept at different storage conditions that is, 8, 25, 40 and 40°C+ 75% RH was found to be increase gradually in the 1st week and then it started to decline continuously till the 28th day with some variations. At the end of the study, pH of the samples of base at 8, 25, 40 and 40°C+ 75% RH was 5.20, 4.60, 4.31 and 4.03, respectively, whereas pH of the samples of formulation kept at 8, 25, 40 and 40°C + 75% RH showed gradual reduction in pH values with slight variations with time. The pH values of samples of formulation kept at 8, 25, 40 and 40°C+ 75% RH were 5.24, 5.70, 5.10 and 5.26 at 28th day, respectively.

By using two-way analysis of variance (ANOVA) technique at 5% level of significance, it was found that the change in pH of different samples of base was not significant at different levels of time and temperature but there was significant difference in change of pH of different samples of formulation at different levels of time and temperature. When least significant difference (LSD)

test was applied to check the individual average effects of the pH of the samples of base at different temperatures with the passage of time by taking average pH values of Zero hour at different temperatures as standard, it gave non-significant changes except 3rd and 4th week where differences were significant. Again, when LSD test was applied to check the individual average effect of the pH of the samples of formulation at different temperatures with the passage of time by taking average pH values of zero hour at different temperatures as standard, it gave significant changes from 48th hour till the study period except the 7th day. From LSD test it was concluded that there was a non-significant change in pH of the samples of base at different storage conditions but significant changes were observed in pH of the samples of formulation at different storage conditions with the passage of time. The decrease in pH of the formulation at different storage conditions might be due to the oxidation of paraffin oil which produces aldehydes and organic acids (Raymond et al., 2003).

Dermatological tests

Skin melanin

The major source of color in human skin derives from the presence within the epidermis of specialized melanin bearing organelles, the melanosomes. Melanosomes synthesized by melanocytes are acquired by keratinocytes and transported within them to the epidermal surface. Tanning of the human skin on exposure to ultraviolet light results from increased amount of melanin within the epidermis (Quevedo et al., 1975).

Polyphenol oxidase, also known as tyrosinase is widely distributed in microorganisms, animals, and plants and is responsible not only for browning in plants but also for melanization in animals. The commercial aldehyde, trans, cis-2, 6-nonadienal, described as a major volatile compound of cucumber, was characterized as a non-competitive inhibitor against 4- tert-butylcatechol oxidetion by mushroom tyrosinase (Fernando et al., 2003). Cucurbitacin D and 23, 24-dihydrocucurbitacin D found in cucumber extract are also responsible for the inhibition of tyrosinase and melanin synthesis (Jian et al., 2005).

In this study, the effect of the base and the formulation on the production of skin melanin was examined. The amount of melanin was measured for 4 weeks at different time intervals in each individual after application of base and formulation and it was found that the base increased the melanin contents in the skin irregularly till the end of the 28th day while the formulation increased the melanin contents in the 1st week but then decreased it gradually throughout the study period. With the help of ANOVA test, it was found that the base and formulation produced a non-significant effects on skin melanin content in volunteers. With the help of paired sample t-test, no significant differences were observed between the melanin effects of base and the formulation throughout the study period.

This showed that the two creams, that is, the formulation and the base, have different effects on melanin but these differences are statistically non-significant for 28 days. It was concluded that the decreased skin melanin content after application of formulation may be attributed to the antioxidant activity of cucumber extract which is rich in vitamins, especially vitamin C (Claudia, 1992) and aldehyde, trans, cis-2,6-nonadienal, a volatile component that causes inhibition of tyrosinase activity (Fernando et al., 2003) and cucurbitacin D and 23, 24-dihydrocucurbitacin D found in cucumber which cause inhibition of tyrosinase and melanin synthesis (Jian et al., 2005) thus inhibiting melanogenesis.

Skin erythema

For confirming the safety of cosmetics, the important point is that cosmetics must not cause any contact dermatitis when applied to the skin. The cause of contact dermatitis is not always due to cosmetic ingredients. Even if the safety of cosmetics is verified, it is known that environmental conditions such as temperature and humidity, misuse by the consumer and the physical conditions may all cause contact dermatitis. Skin irritation is caused by the direct toxicity of chemicals on cells or blood vessels in the skin and is different from contact allergy which is caused by immune response (Naveed, 2001).

In this study, irritation was constantly monitored every week for the base and the formulation throughout the period of application. It was found that erythema contents decreased in an irregular pattern after the application of base throughout the study period. Whereas, after the application of formulation, erythema contents were slightly increased as the study progressed. With the help of ANOVA test, it was found that the base and formulation produced non-significant effects on skin erythema at different time intervals and with the help of paired sample t-test, it was evident that there was no significant variation in irritation with respect to base and formulation throughout the study period.

It was concluded that the base decreased while formulation slightly increased the erythema contents of skin at the end of study period and overall effect of formulation on skin erythema was non-significant, so it can be used safely without any significant skin irritation. As cucumber extract is rich in vitamins (Claudia, 1992), it contains vitamin A too. So the increase in the erythema contents in case of formulation may be attributed to irritation caused by isotretinoin (Hughes et al., 1992) which is an important metabolite of vitamin A (Kathryn, 1998). The assumption for presence of isotretinoin is further strengthened by the reduction in sebum contents.

Skin moisture content

The moisturizing treatment involves repairing the skin barrier, retaining or increasing water content, reducing TEWL, restoring the lipid barriers' ability to attract, hold and redistribute water, and maintaining skin integrity and appearance. Formulation of this study contained cucumber extract as an active ingredient which is rich in vitamins, especially vitamin C (Claudia, 1992). Vitamin C is known to increase the collagen fibers in the dermis. With the increase in collagen, the hydration conditions in the dermis are improved (Padayatty and Levine, 2001). In addition to this, extract also contains cucurbitacins, the bitter principles of the cucurbitaceae (Gaofeng et al., 2006) which are known for their non-specific cytotoxicity, and very little is known about the mechanism of the effect of cucurbitacins at the cellular and molecular level (Jian et al., 2005). These may disrupt the super facial layer of skin. That is, stratum corneum, thus reducing the moisture contents.

In this study, it was found that there was a slight increase in moisture values at the 1st and 2nd week after the application of the base and a very slight decrease was observed at the 3rd and 4th week; however after the application of formulation, the increase in skin moisture content was limited to the 1st week only, after this, it decreased at the 2nd week and then remains almost same throughout the study period. With the help of ANOVA test, it was found that the base showed a non-significant change with respect to the basic values whereas the formulation showed a significant variation throughout the study period of 28 days. By LSD test for both base and formulation, it was found that significant change in moisture content was observed only after the 1st week of application. Using paired sample t-test, it was evident that insignificant differences in the moisture values were observed after application of base and formulation throughout the study period. The significant reduction in moisture after application of formulation may be due to cucurbitacin components of the cucumber, as cytotoxicity of cucurbitacins is known before 1800 AD, but very little is known about the mechanism of the effect of cucurbitacins at the cellular and molecular level (Jian et al., 2005). They may have disrupted the stratum corneum so that water evaporated from the skin at higher rates causing reduction in skin moisture. This argument is further strengthened by the fact that TEWL also increased significantly after application of formulation in this study.

Skin sebum content

Sebum, the product of sebaceous glands, is a complex of various lipids that are thought to act as an epidermal and/or follicular lubricant (Robert et al., 2000). Sebum production is measured using a special opalescent plastic film, which becomes transparent when it is in contact with sebum lipids. The device relies on a probe which presses a piece of special film on the skin for a measured length of time. The sebum is adsorbed on this film like ink on the blotting paper and the film becomes transparent. The probe is then placed into the device which radiates a light beam onto the film. A metal mirror behind the film reflects the beam back again through the film and then into an instrument called a photomultiplier, which measures the amount of light in the beam. The more sebum on the skin, the more transparent is the film and the greater the amount of light reflected.

In this study, the effects of the base and formulation on the sebum contents of human cheeks were investigated. Sebum was measured every week in all the individuals. It was found that the base increased sebum contents in the 1st and 4th week of study period but decreased in the 2nd and 3rd week, while the formulation showed a gradual reduction in sebum contents in a regular manner as the study continued from the 1st to 4th week. With the help of ANOVA test, it was evident that there was a significant effect of base and formulation on skin sebum throughout the study period. By applying LSD test, it was evident that non-significant changes in sebum contents were observed at different time intervals after application of base while significant changes were observed after application of formulation except the 1st week of study. With paired sample t-test, it was found that the base and formulation showed insignificant variations regarding the skin sebum content after the 1st, 2nd and 3rd week while significant variations were shown after the 4th week of study.

It is concluded that increase in sebum contents after the application of base may be attributed to the oily nature of w/o emulsion having a thick viscous oily liquid, that is, the paraffin oil (Henriette, 1995) while significant reduction in sebum after application may be because of isotretinoin, a natural metabolite of vitamin A (Kathryn, 1998), which is most effective in reducing sebaceous gland size by decreasing proliferation of basal sebocytes and in suppressing sebum production up to 90% by inhibiting sebaceous lipid synthesis (Christos, 2006). The assumption for presence of isotretinoin was further strengthened by irritation observed in this study.

Trans epidermal water loss (TEWL)

TEWL is the outward diffusion of water through skin (Jackie and Howard, 2005). TEWL is a measure of cutaneous barrier function and also reflects skin water content (Ostlere et al., 1994). An increase in TEWL reflects an impairment of the water barrier. TEWL measurements allow parametric evaluation of the effect of barrier creams against irritants and characterization of skin functionality in clinical dermatitis and in irritant and allergic patch test reactions. TEWL measurements can

be affected by the anatomical site, sweating, skin surface temperature, inter- and intra-individual variation, air convection, ambient air temperature, ambient air humidity, and instrument related variables [Jackie, Howard, 2005).

In this study, it was found that there was increase in TEWL values after the application of base having the greatest value after the 1st week then gradual reduction in loss, and after formulation, there was increase in TEWL after the 1st, 3rd and 4th week but decrease in the 2nd week of study. With the help of ANOVA test, it was found that changes in TEWL produced by formulation were significant but non-significant for base during a period of 4 week study. By applying LSD test, it was observered that in both cases, that is, base and formulation change in TEWL, values became significant after the 1st week of application. Using paired sample t-test, it was found that there was significant variation in TEWL with respect to base and formulation after the 2nd week of study but non-significant for other periods.

The significant increase in TEWL after application of formulation may also be due to cucurbitacin components of the cucumber. They may cause disruption of the stratum corneum thus increasing the rate of water loss from the skin. This argument was further supported by the fact that skin moisture also decreased significantly after application of formulation in this study.

Conclusion

This project was a preliminary step to explore the hidden potential of cucumber for skin rejuvenation in the form of topical cream**.** From our findings we concluded that a stable topical cream (w/o emulsion) containing cucumber extract can produce a pronounced decrease in melanin content of the skin showing that the formulation has skin whitening effects. The cream produced a pronounced decrease in skin sebum content showing that the formulation has anti-acne effects. The formulation was also observed to decrease skin moisture content and increase TEWL which strengthens the anti-acne effects. However, further a targeted study needs to be conducted in future in patients with freckles/melasma, acne, psoriasis as well in people with dry wrinkled skin to evaluate this product for the cosmetic market. Our investigations have proved to be promising in terms of future potential applications of cucumber extract, as skin-care products, cosmetics and/ or pharmaceutical preparations owing to these properties.

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